ENFERMEDAD MÍNIMA RESIDUAL:

DETECCION POR CITOMETRIA DE FLUJO EN LLA PEDIATRICA.

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GRUPO RIOPLATENSE DE CITOMETRIA DE FLUJO

ENFERMEDAD MINIMA RESIDUAL (EMR)

DEFINICION:

Presencia de células leucémicas indetectables a la microscopía óptica, que pueden expandirse en cualquier momento y producir una recaída de la enfermedad.

Métodos "convencionales" disponibles:

CITOMETRÍA DE FLUJO MULTIPARAMÉTRICA

BIOLOGÍA MOLECULAR (RqPCR-NGS)

EMR: MÉTODOS DISPONIBLES

	Citometría de flujo (CF)	Biología molecular (Rq PCR)
Fundamento	-Inmunofenotipo aberrante.	-Rearreglos de Ig/TCR. -Genes de fusión
Ventajas	-Cuantificación directa. -Menor cantidad de células. -Más económico. -Resultados más rápidos. -Evaluación celularidad global y viabilidad de la médula.	-Mayor sensibilidad. -Target paciente específico.
Limitaciones	-Posibles falsos negativos por cambios inmunofenotípicos. -Menor sensibilidad.	-Posibles falsos negativos por pérdida del target. -Mayor cantidad de células. -Muy laborioso.

Detección de eventos poco frecuentes por CF depende básicamente de dos variables:

* Diferencia fenotípica entre blastos y células normales
* Número de células analizadas.

SENSIBILIDAD

*En condiciones adecuadas 1 en 10^3 a 10^4 (0.1-0.01%)

*En laboratorios entrenados, condiciones adecuadas y estandarizadas del citómetro y adquiriendo ≥1x10^6cél:

1 en 10^4 a 10^5 (0.01 a 0.001%)

Cytometry Part B (Clinical Cytometry) 74B:331–340 (2008)

Original Articles

Standardization of Flow Cytometric Minimal Residual Disease Evaluation in Acute Lymphoblastic Leukemia: Multicentric Assessment Is Feasible

Michael Norbert Dworzak,[1*] Giuseppe Gaipa,[2] Richard Ratei,[3] Marinella Veltroni,[4,5] Angela Schumich,[1] Oscar Maglia,[2] Leonid Karawajew,[3] Allessandra Benetello,[4] Ulrike Pötschger,[1] Zvenyslava Husak,[1] Helmut Gadner,[1] Andrea Biondi,[2] Wolf-Dieter Ludwig,[3] and Giuseppe Basso[4]

[1]Children's Cancer Research Institute and St. Anna Children's Hospital, Vienna, Austria

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[3]Department of Hematology, Oncology and Tumor Immunology, Robert-Roessle-Clinic at the HELIOS Klinikum Berlin, Charité Medical School, Berlin, Germany

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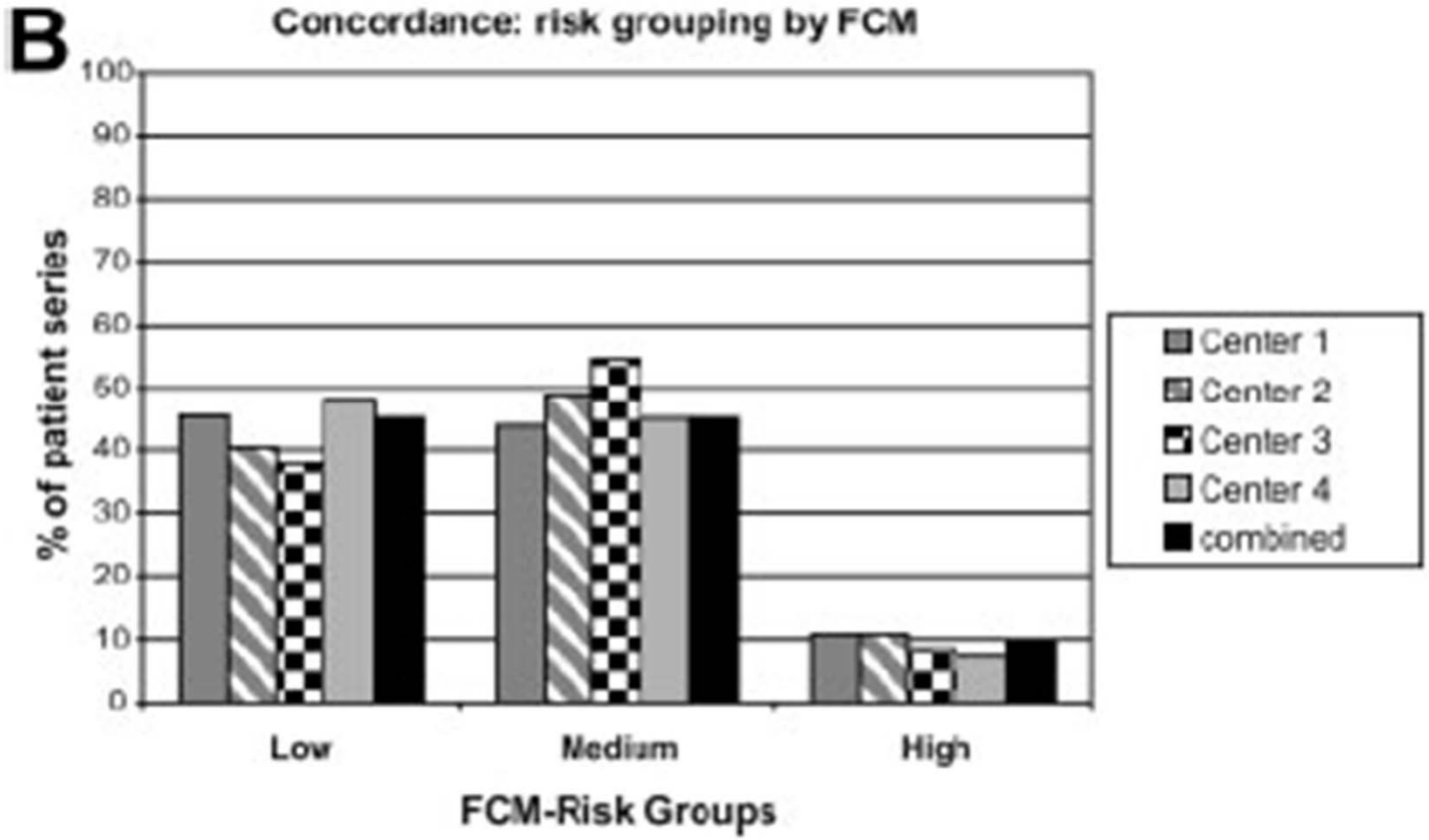
[5]Department of Pediatrics, University of Florence, Florence, Italy

Background: Single-laboratory experience showed that flow cytometric (FCM) assessment of minimal residual disease (MRD) in acute lymphoblastic leukemia (ALL) is feasible in most patients and gives independent prognostic information. It is, however, not known whether FCM analysis can reliably be standardized for multicentric application.

Methods: An extensive standardization program was installed in four collaborating laboratories, which study FCM-MRD in children treated with the AIEOP-BFM-ALL 2000 protocol. This included methodological alignment, continuous quality monitoring, as well as personnel education by exchange and performance feed-back.

Results: Blinded inter-laboratory tests of list-mode data interpretation concordance (n = 202 blood and bone marrow samples from follow-up during induction of 31 randomly selected patients of a total series of n = 395) showed a very high degree of inter-rater agreement among the four centers despite differences in cytometers and software usage (intraclass correlation coefficient [ICC] 0.979 based on n = 800 single values). Lower concordance was reached with amounts of MRD below 0.1%. Comparing data from sample exchange experiments (n = 42 samples; ICC 0.98) and from independent patient cohorts from the four centers (regarding positive samples per time-point of follow-up as well as risk estimates) concordance was also good.

Conclusion: MRD-evaluation by FCM in ALL can be standardized for reliable multicentric assessment in large trials. © 2008 Clinical Cytometry Society



Dworzak 2008

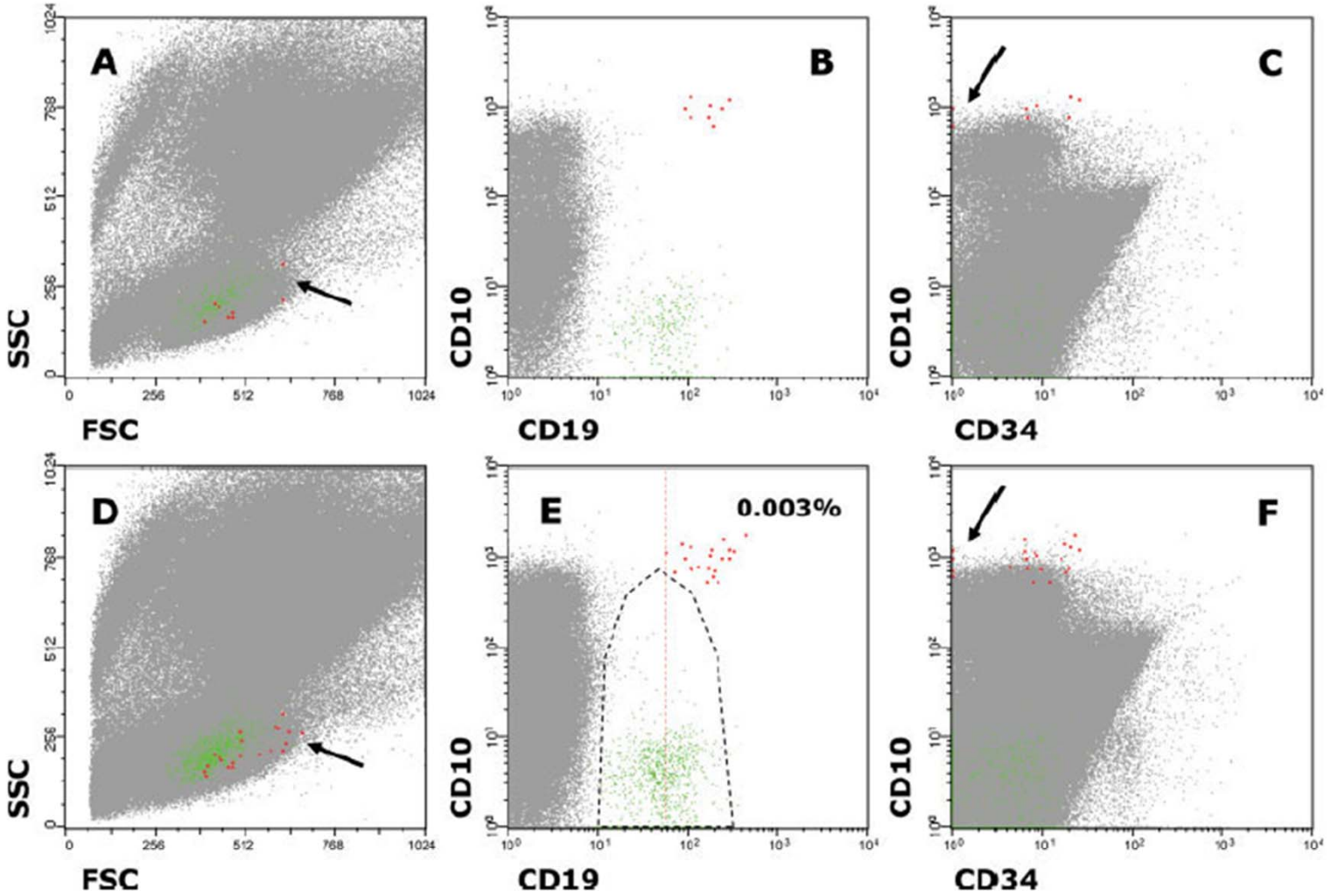


FIG. 5. Dot plot chart exemplifying the impact of increased cell input and acquisition on sensitivity in flow cytometric MRD analysis (**A–C**, 300,000 events; **D–F**, 750,000 events of a Day-15 BM sample of a patient treated for BCP-ALL; plot organization as in Fig. 1). Note the eight (red) events forming a MRD-suspect cluster in (B). According to study-definitions (MRD positive is $\geq$10 clustered events with lymphoid scattering properties and leukemia-associated immunophenotypic characteristics), this sample had to be considered negative. However, by acquiring more events, the same sample can unambiguously be estimated MRD-positive: 21 clustered $CD10^{++}$ events in (E) with leukemia-associated CD19 overexpression (note difference between MRD [red] and normal B cells [green]). This demonstrates that populations of even less than 10 events potentially could be considered as "positive" if forming "perfect" clusters. However, different from the expression of CD10 and CD19 on leukemic cells, complementary markers (like CD34, as well as CD20, CD45, CD11a) are frequently less homogeneously expressed (note arrows on skewed MRD events in A and D regarding CD34; C and F: FSC). Hence, the requirement of at least 10 clustered events to consider a sample definitely MRD-positive seems more appropriate. It also becomes clear from this example that by increasing the cell acquisition, the quantitative reliability at the level around 0.01% and the concordance between centers will improve.

LYMPHOID NEOPLASIA

Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia

Prisca Theunissen,[1,*] Ester Mejstrikova,[2,*] Lukasz Sedek,[3] Alita J. van der Sluijs-Gelling,[4] Giuseppe Gaipa,[5] Marius Bartels,[6] Elaine Sobral da Costa,[7] Michaela Kotrová,[2] Michaela Novakova,[2,6] Edwin Sonneveld,[4] Chiara Buracchi,[5] Paola Bonaccorso,[5] Elen Oliveira,[7] Jeroen G. te Marvelde,[1] Tomasz Szczepanski,[3] Ludovic Lhermitte,[8] Ondrej Hrusak,[2] Quentin Lecrevisse,[9] Georgiana Emilia Grigore,[10] Eva Froňková,[2] Jan Trka,[2] Monika Brüggemann,[6] Alberto Orfao,[9] Jacques J. M. van Dongen,[1] and Vincent H. J. van der Velden,[1] on behalf of the EuroFlow Consortium

Key Points

- Standardized flow cytometry allows highly sensitive MRD measurements in virtually all BCP-ALL patients.
- If sufficient cells are measured (>4 million), flow cytometric MRD analysis is at least as sensitive as current PCR-based MRD methods.

A fully-standardized EuroFlow 8–color antibody panel and laboratory procedure was stepwise designed to measure minimal residual disease (MRD) in B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) patients with a sensitivity of $\leq 10^{-5}$, comparable to real-time quantitative polymerase chain reaction (RQ-PCR)–based MRD detection via antigen-receptor rearrangements. Leukocyte markers and the corresponding antibodies and fluorochromes were selected based on their contribution in separating BCP-ALL cells from normal/regenerating BCP cells in multidimensional principal component analyses. After 5 multicenter design-test-evaluate-redesign phases with a total of 319 BCP-ALL patients at diagnosis, two 8-color antibody tubes were selected, which allowed separation between normal and malignant BCP cells in 99% of studied patients. These 2 tubes were tested with a new erythrocyte bulk-lysis protocol allowing acquisition of high cell numbers in 377 bone marrow follow-up samples of 178 BCP-ALL patients. Comparison with RQ-PCR–based MRD data showed a clear positive relation between the percentage concordant cases and the number of cells acquired. For those samples with >4 million cells acquired, concordant results were obtained in 93% of samples. Most discordances were clarified upon high-throughput sequencing of antigen-receptor rearrangements and blind multicenter reanalysis of flow cytometric data, resulting in an unprecedented concordance of 98% (97% for samples with MRD < 0.01%). In conclusion, the fully standardized EuroFlow BCP-ALL MRD strategy is applicable in >98% of patients with sensitivities at least similar to RQ-PCR ($\leq 10^{-5}$), if sufficient cells (>4 × 10^6, preferably more) are evaluated. (*Blood*. 2017;129(3):347-357)

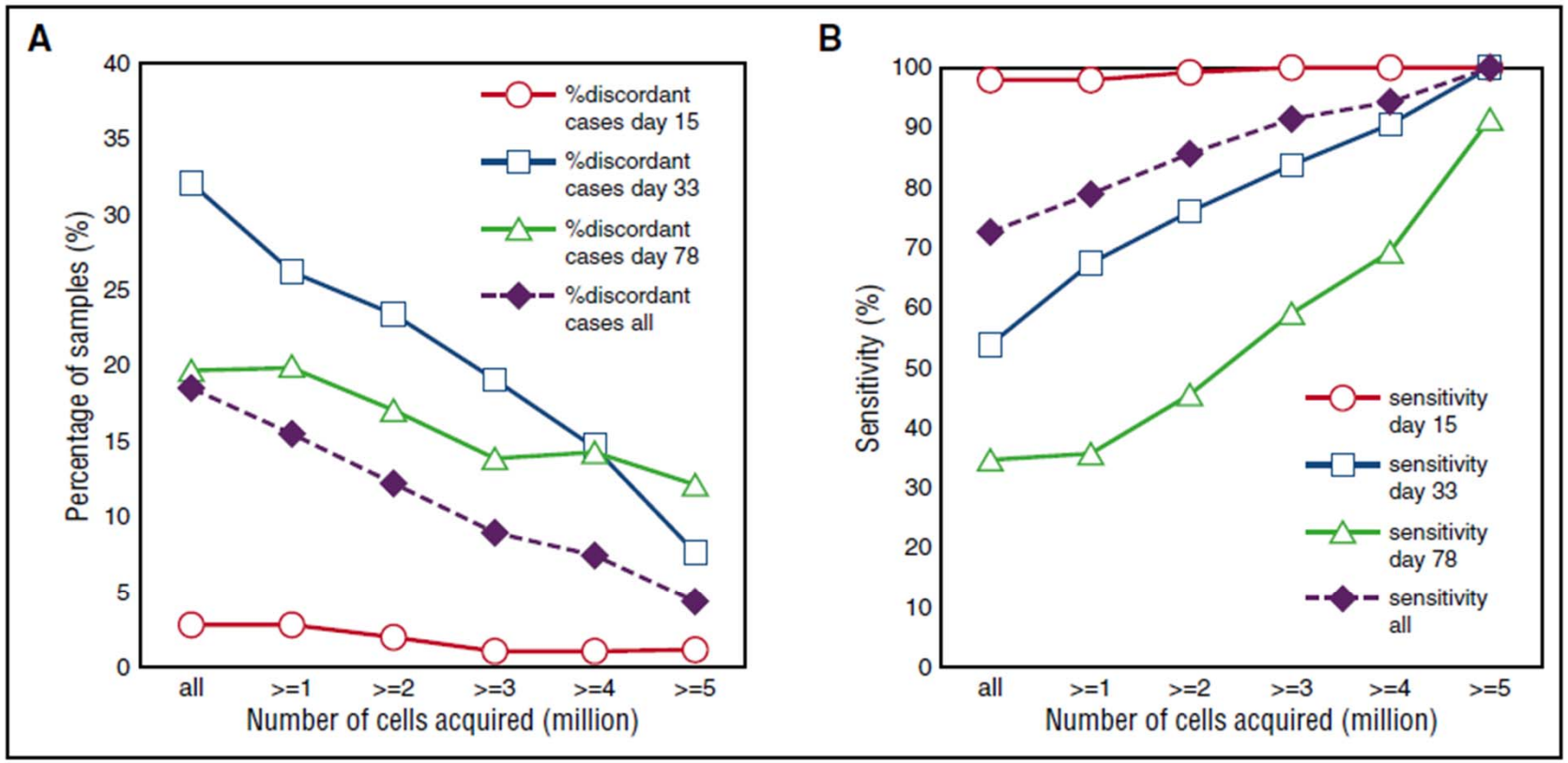


Figure 4. Performance of FCM-MRD vs PCR-based MRD is dependent on the number of acquired cells. (A) The percentage of discordant cases by FCM-MRD and PCR-MRD is shown for individual time points (day 15, day 33, and day 78) as well as for all samples together. Data are presented for variable numbers of acquired cells: all samples (independent of cell number) and samples with at least 1, 2, 3, 4, or 5 million cells acquired. (B) The sensitivity of FCM-MRD relative to PCR-MRD is shown for individual time points (day 15, day 33, and day 78) as well as for all samples together. Data are presented for variable numbers of acquired cells: all samples (independent of cell number; n = 377) and samples with at least 1 (n = 330), 2 (n = 287), 3 (n = 255), 4 (n = 227), or 5 million cells (n = 191) acquired. Sensitivity is calculated as the number of samples positive by both FCM and PCR divided by the total number of samples positive by PCR (ie, the reference method).

La identificación de los blastos se basa en que su inmunofenotipo es diferente al de las células normales de médula ósea.

Que debemos saber antes de hacer EMR:

INDISPENSABLE:
Inmunofenotipo de las cél. hematopoyéticas normales de médula ósea.

IDEAL pero no excluyente:
Inmunofenotipo de las células leucémicas o blastos en su presentación .

Evaluación fenotipo:

LLA-B:

- **Gate primario de análisis: SSC/CD19**

En lo CD19+ evaluar CD10 / CD34 / CD45 /CD20 / CD58 /CD38 fundamentalmente. También CD11a / CD123 / CD81, CD66c etc

LLA-T:

- **Gate primario de análisis: SSC/CD7**

En lo CD7+ evaluar CD5 /CD3^{sup} e ^{intracito}/CD4 /CD8 /CD99 /Tdt /CD34 /CD45, etc

En ambos casos se debe evaluar la ausencia-presencia de los marcadores, el nivel de intensidad de expresión (IMF), asincronismos madurativos, expresión ectópica de antígenos, etc.

Panel BFM-ALLIC-09 para EMR LLA-B

Junio 2014 Panel 6 col

1.-CD10/CD19/CD20/CD45/CD34/SYTO

2.-CD10/CD19/CD20/CD45/CD58/CD38

3.- opcional: incluir otro Ac al backbone por ej CD11a (sólo PE), CD66c, CD123, CRLF2, etc)

Junio 2014 Panel 8 col
CD10/CD19/CD20/CD45/CD34/CD58/CD38/syto

Panel BFM-ALLIC-09 para EMR LLA-T

Panel 6 col Junio 2014

1.-CD3/CD5/CD7/CD45/CD99/SYTO

2.-CD3intracito/CD3s/CD7/CD45/CD99/Tdt

3.- CD3/CD4/CD5/CD7/CD8/CD45

Opcional TCR-CD16-CD56-CD34-CD117 agregado al backbone CD3-CD5-CD7-CD45

Panel 8 col Junio 2014

CD3-CD4-CD5-CD7-CD8-CD45-CD99-syto

cyCD3-sCD3-CD5-CD7-CD45-CD99-Tdt-syto

La presencia de células con fenotipo inmaduro en M.O es sinónimo de EMR positiva?

Depende...

- Del linaje de las mismas
- De la etapa de tratamiento

En cuanto al linaje:

La ontogenia T ocurre exclusivamente en timo:

La presencia de precursores T en médula es patológico.

La ontogenia B es en médula ósea:

Fundamental definir si, en caso de encontrarse células con fenotipo inmaduro, se trata de precursores normales (hematogonias) o de células patológicas.

En cuanto a la etapa del tratamiento:

Es fundamental conocer en que fase del tratamiento está el paciente al momento de la toma de muestra, especialmente en LLA-B.

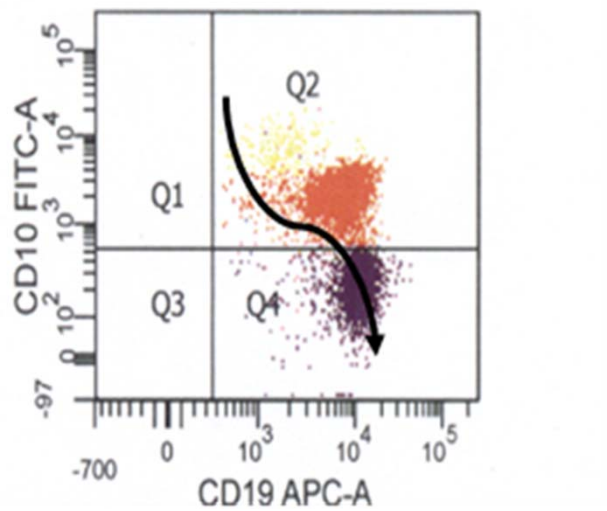
Recuperación del linaje B durante el tto tipo ALLIC-BFM.

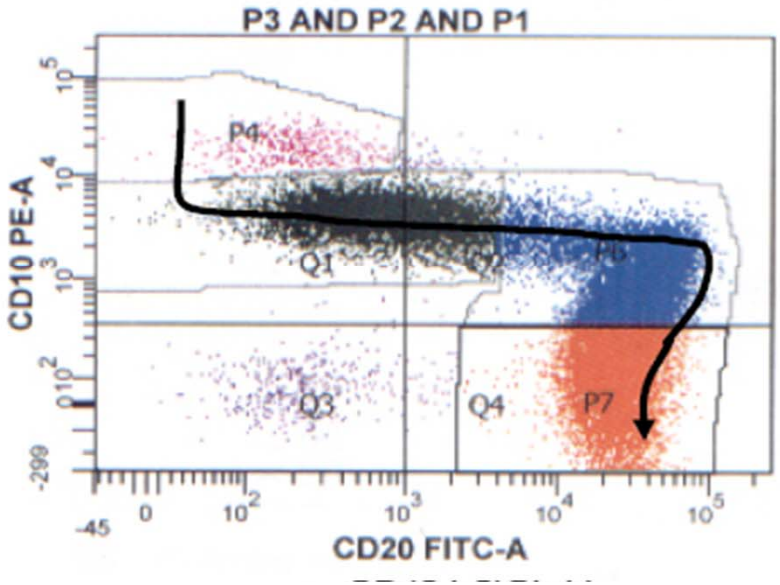
- **Día 15:** Ausencia de hematogonias. La presencia de CD19+CD10+y/o CD34+ es indicador de EMR positiva. (Coustan Smith, Blood 2006;108)
- **Día 33:** no hay aún regeneración normal B. Sólo LB maduros c/s plasmocitos, raramente hematogonias.
- **Semana 12 o posterior:** hematogonias c/s plasmocitos c/s LB maduros.
- **Pre Tx:** suele ser muy bajo el % CD19+ y puede haber hematogonias.

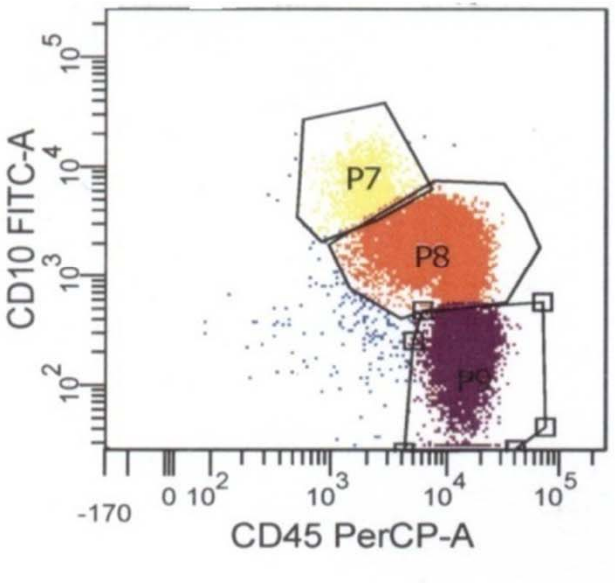
Perfil normal de expresión antigénica en MO.

Linaje linfoide B

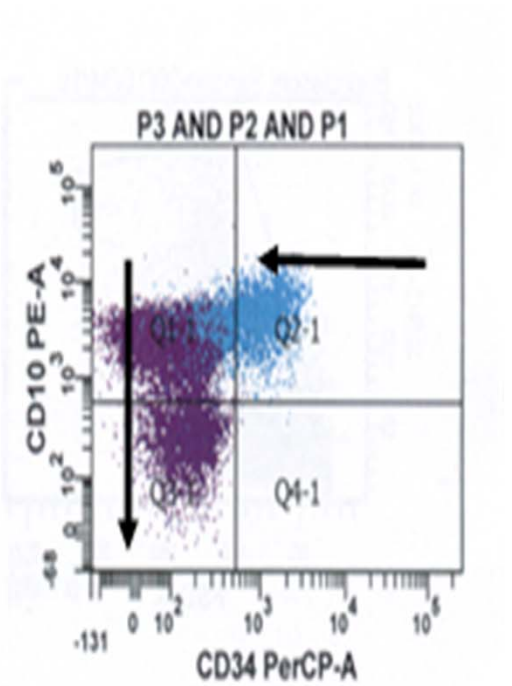
Gate en SSC-CD19+

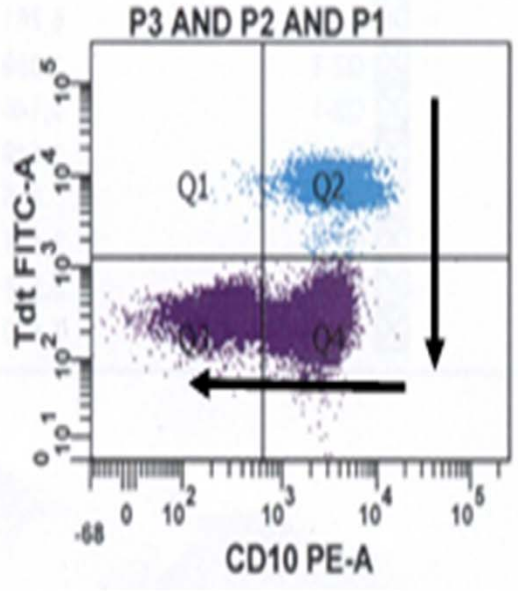


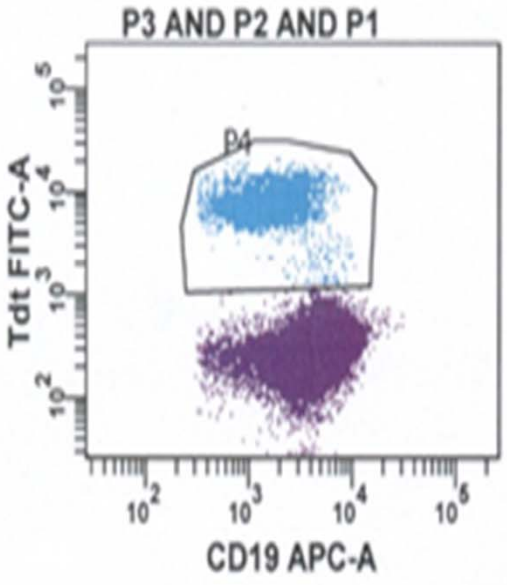


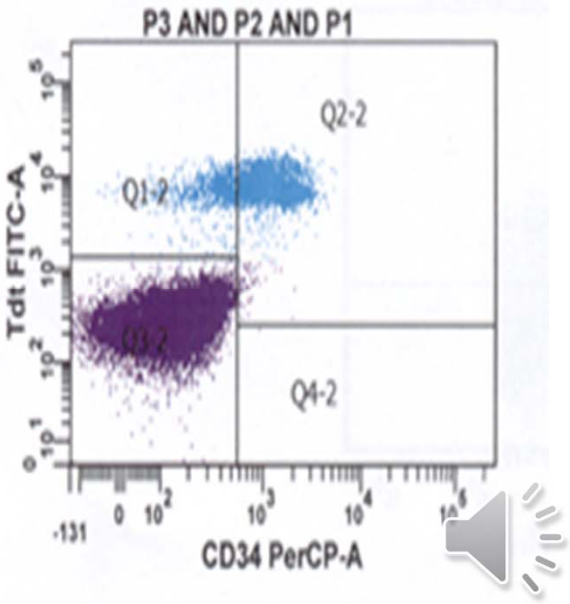


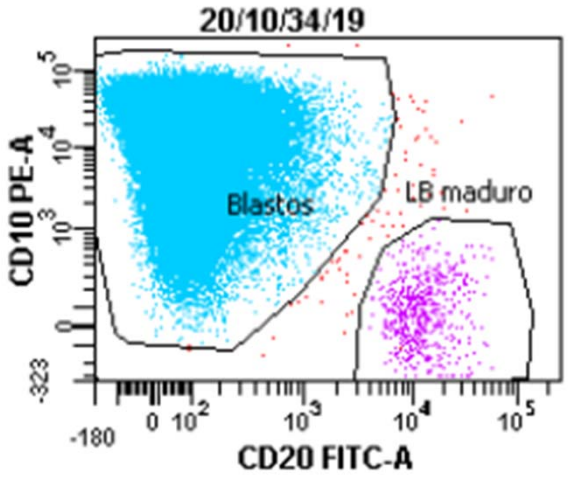
Más perfiles madurativos normales de linaje B en médula ósea:



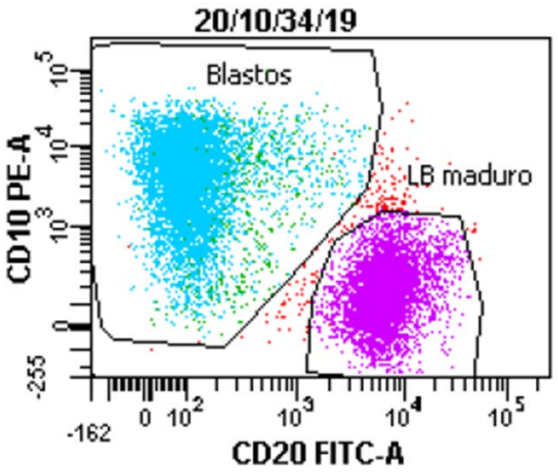




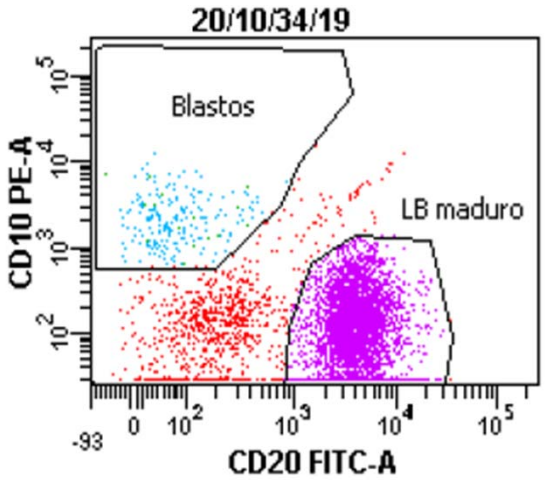




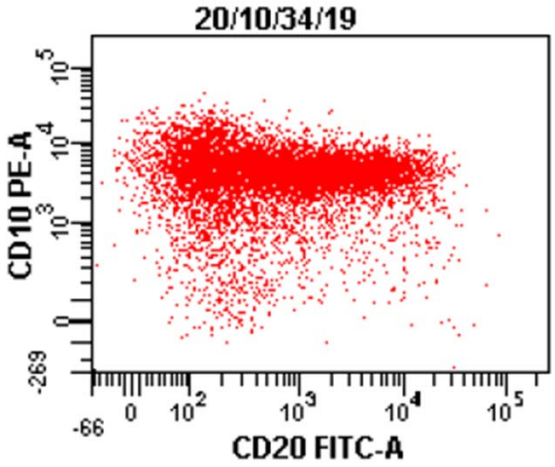
Diagnóstico



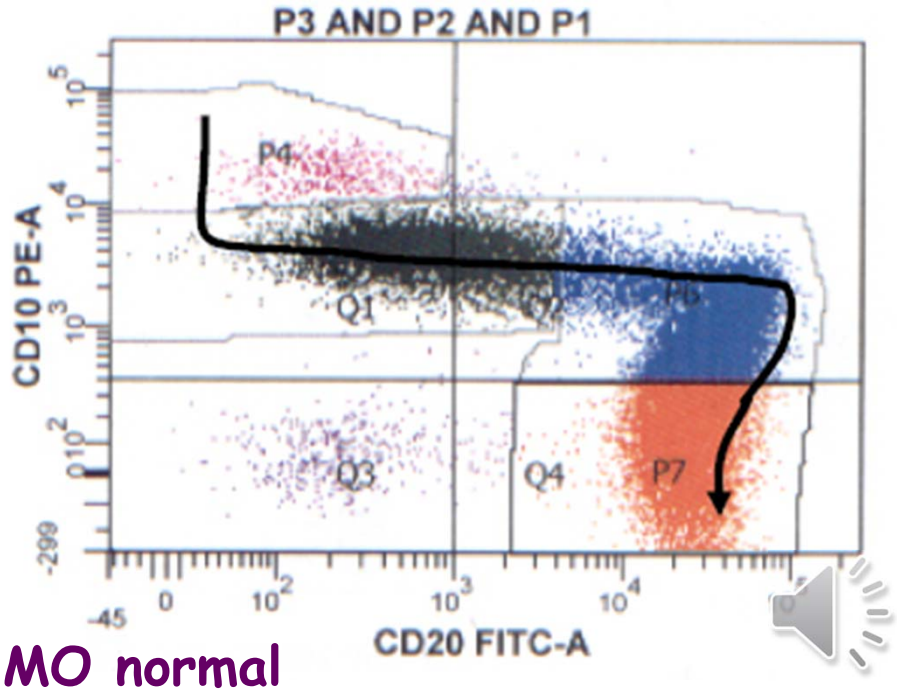
Día 15



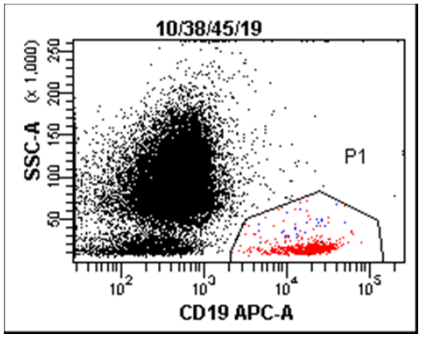
Día 33

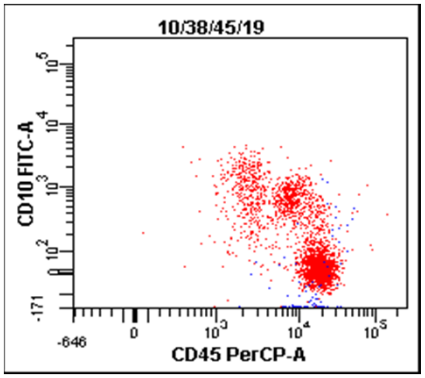


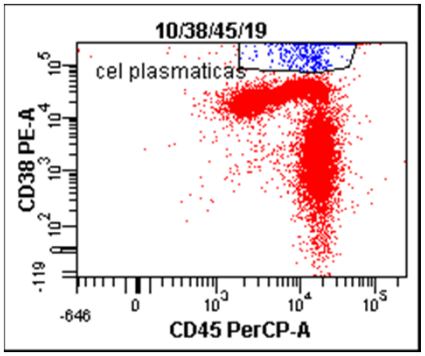
Sem 12



MO normal

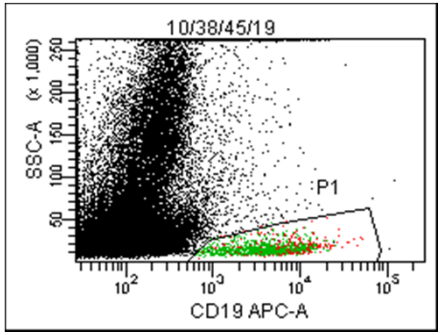


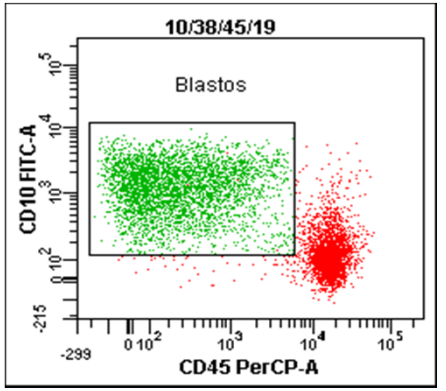


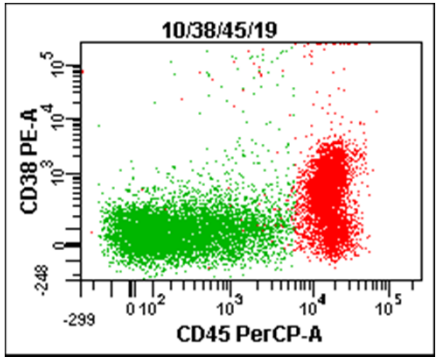


M.O. Normal
linaje B
regenerativo

LLA-B día 15

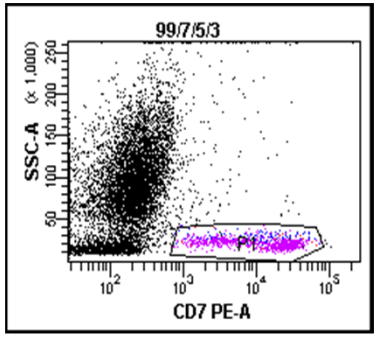


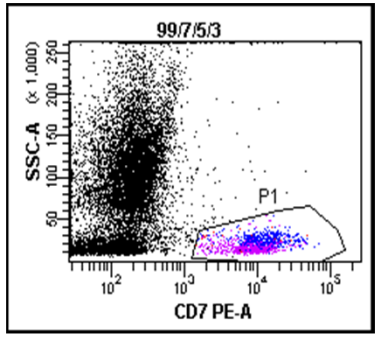


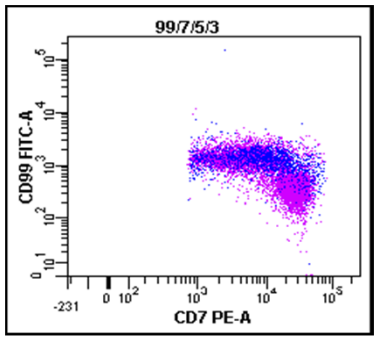


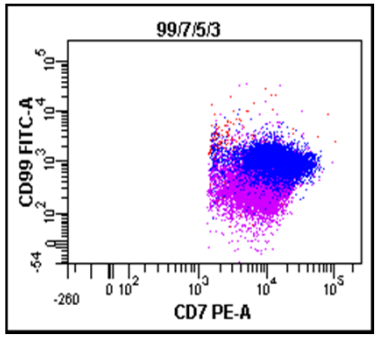
Blastos CD45 negativo-tenue y CD38 negativo, ambos hallazgos aberrantes.

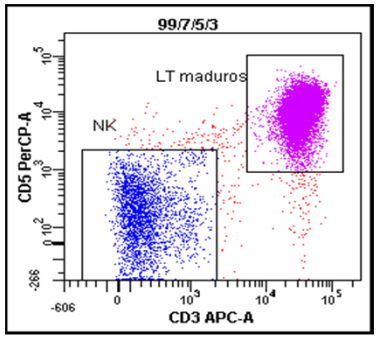
M.O. Normal

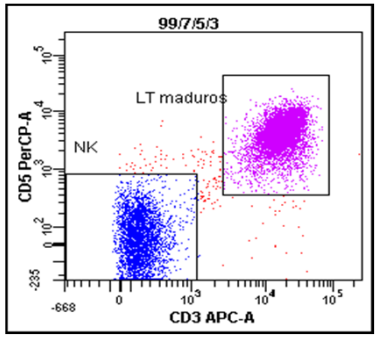








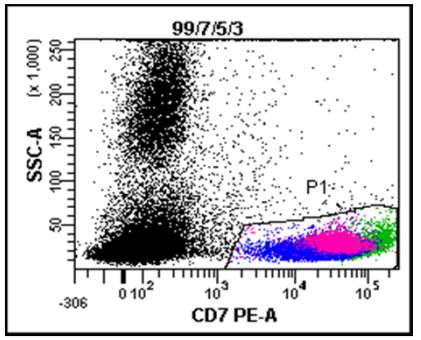


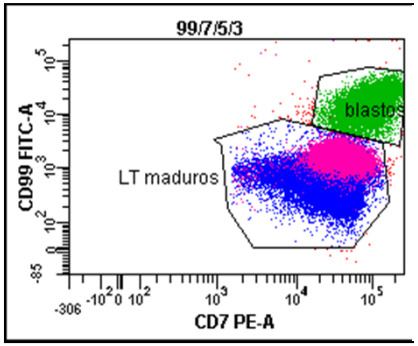


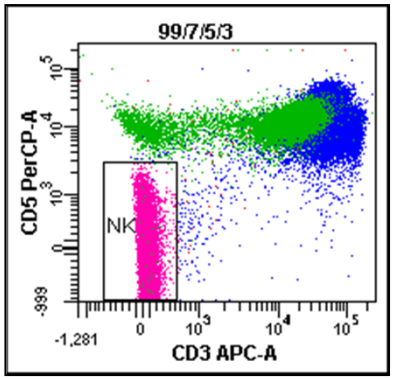
Normal #1

Normal #2

LLA-T dia 15







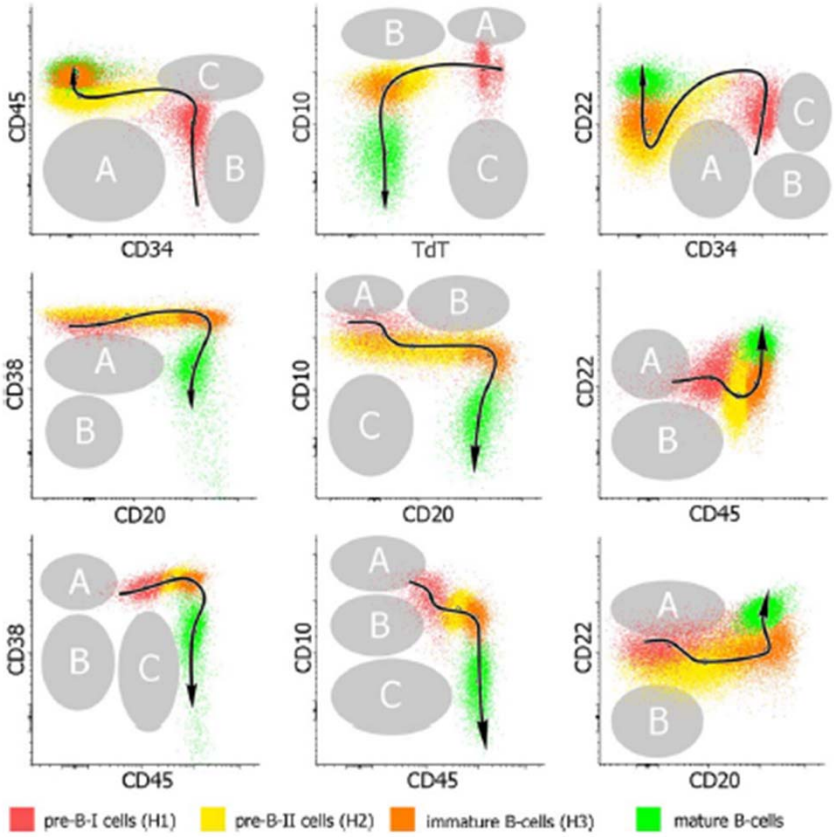
Los blastos muestran > IMF de CD99 y CD7 que los normales y CD3 superficie parcial

Cytometry Part B (Clinical Cytometry) 86B:329–339 (2014)

Original Article

The Immunophenotypes of Blast Cells in B-Cell Precursor Acute Lymphoblastic Leukemia: How Different Are They from Their Normal Counterparts?

Ł. Sędek,[1*] J. Bulsa,[1] A. Sonsala,[1] M. Twardoch,[1] M. Wieczorek,[2] I. Malinowska,[3] K. Derwich,[4] M. Niedźwiecki,[5] G. Sobol-Milejska,[6] J. R. Kowalczyk,[7] B. Mazur,[8] and T. Szczepański[1]



Cytometry Part B (Clinical Cytometry) 74B:150–155 (2008)

Prednisone Induces Immunophenotypic Modulation of CD10 and CD34 in Nonapoptotic B-Cell Precursor Acute Lymphoblastic Leukemia Cells

Giuseppe Gaipa,[1*] Giuseppe Basso,[2] Sara Aliprandi,[1] Maddalena Migliavacca,[1] Cristina Vallinoto,[1] Oscar Maglia,[1] Andrea Faini,[3] Marinella Veltroni,[4] Dzvenyslava Husak,[5] Angela Schumich,[5] Richard Ratei,[6] Andrea Biondi,[1] and Michael N. Dworzak;[5] on behalf of the I-BFM-ALL-FCM-MRD-Study Group

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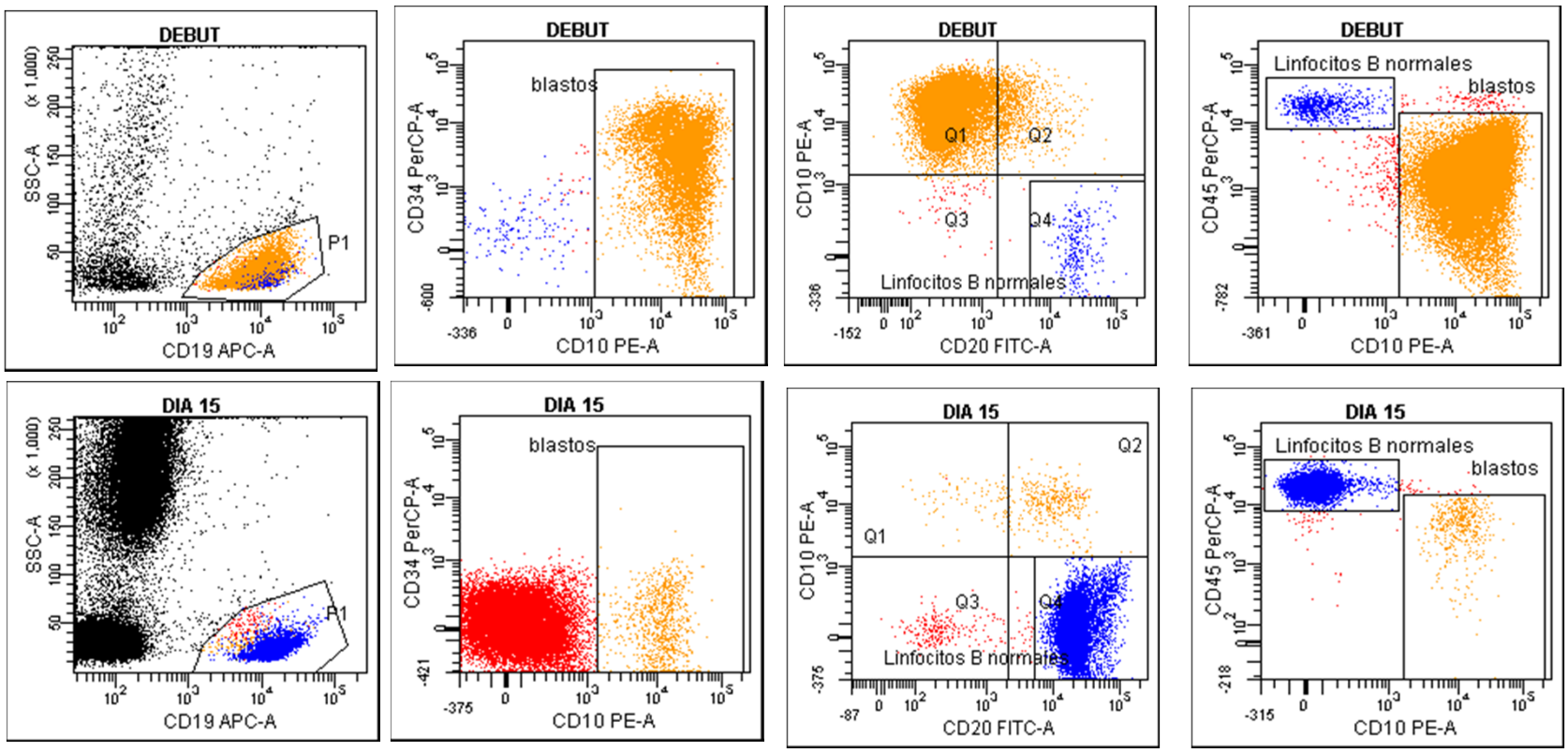
CAMBIOS INMUNOFENOTÍPICOS: 16/31 casos(2010) (52%)



‹CD34 ›CD20 ›CD45

Cytometry Part B (Clinical Cytometry) 78B:139–146 (2010)

Original Articles

Immaturity Associated Antigens Are Lost During Induction for T Cell Lymphoblastic Leukemia: Implications for Minimal Residual Disease Detection

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Background: Induction chemotherapy for acute leukemia often leads to antigenic shifts in residual abnormal blast populations. Studies in precursor B cell ALL (B-ALL) have demonstrated that chemotherapy commonly results in the loss of antigens associated with immaturity, limiting their utility for minimal residual disease (MRD) detection. Little information is available about the stability of these antigens in precursor T cell ALL (T-ALL) though it is presumed that CD99 and terminal deoxynucleotidyl transferase (TdT) are highly informative based on limited studies.

Methods: In a longitudinal investigation, we explored patterns of lineage specific and immaturity-associated antigens in T-ALL in a large cohort of patients treated under the multicenter Children's Oncology Group (COG) protocol. All samples were analyzed using multicolor flow cytometry in a standardized fashion at a single institution.

Results: We report that markers of immaturity particularly, TdT and CD99, dramatically decline on leukemic blasts during therapy. CD34 and CD10 expression is confined to a minority of pretreatment samples and is also not stable. In contrast, lineage-associated markers including CD2, CD3, CD4, CD5, CD7, and CD8 failed to show significant trends.

Conclusions: Our study strongly argues for expansion of immunophenotyping panels for T-ALL MRD to decrease reliance on immature antigens. This study represents the first demonstration of consistent immunophenotypic shifts in T-ALL. © 2010 Clinical Cytometry Society

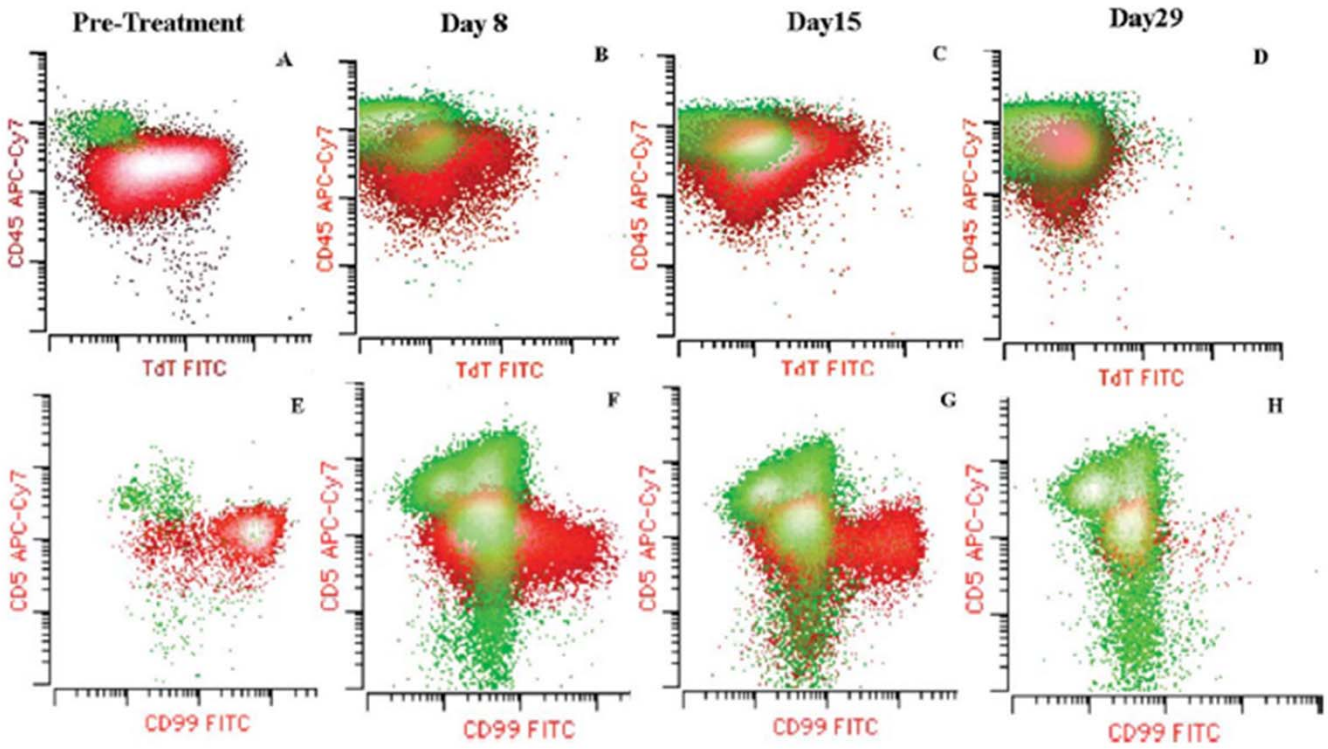

FIG. 1. Typical expression profile of CD99 (**A–D**) and TdT (**E–H**) over the 29 days postinduction chemotherapy. Normal T cell population (green) is used as an internal negative control for expression of CD99 and TdT by the abnormal T lymphoblasts (red). At day 0 (A and E), a majority of cells express CD99 and TdT. Expression of both antigens declines over time despite persistence of abnormal blast populations at days 8 (B and F), 15 (C and G), and 29 (D and H). The abnormal population is identified in each specimen by the abnormally decreased expression of CD45 and CD5 with expression of CD7 in a multiparametric assessment. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

PORQUE HACER EMR EN LLA?

Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies

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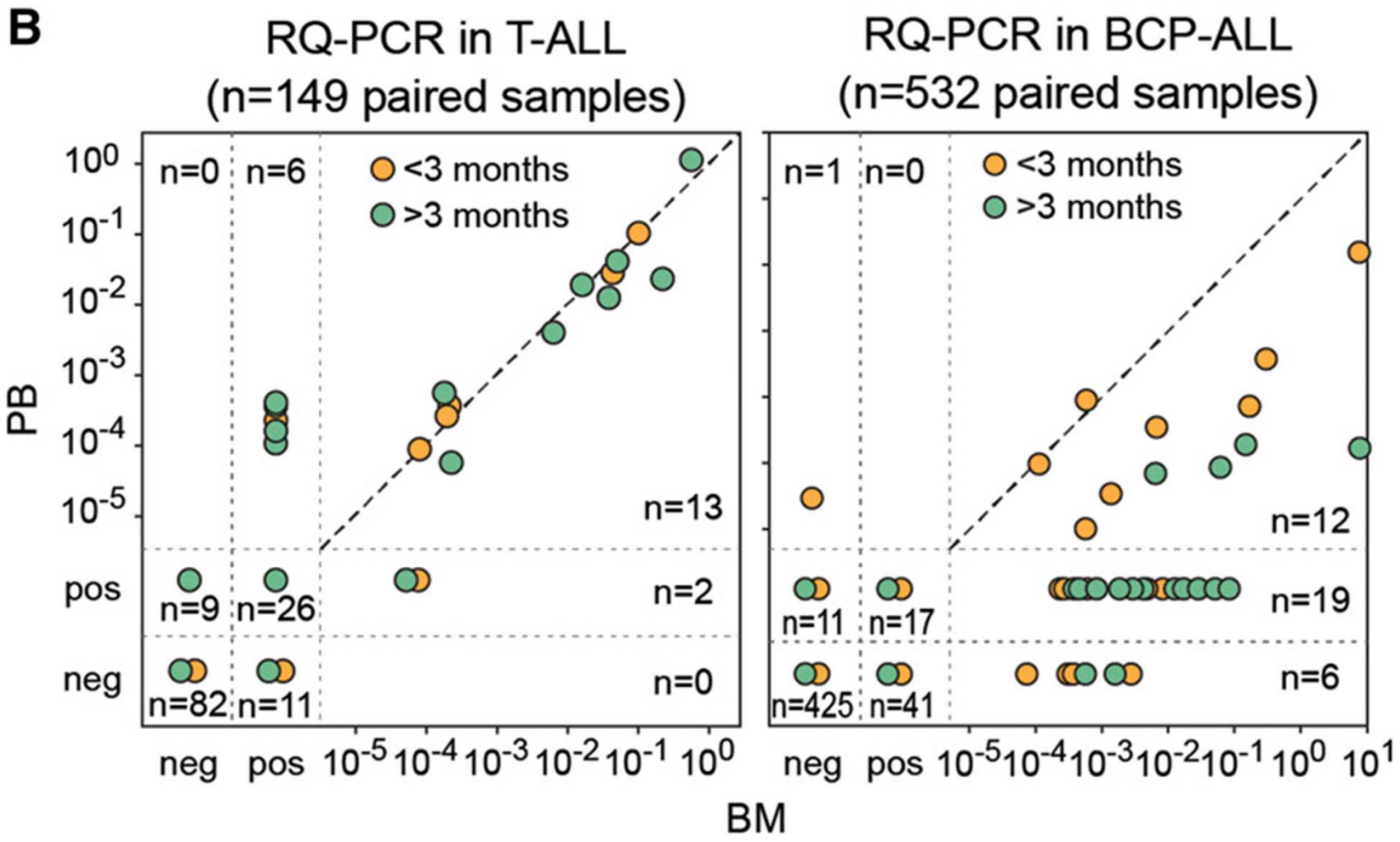
Monitoring of minimal residual disease (MRD) has become routine clinical practice in frontline treatment of virtually all childhood acute lymphoblastic leukemia (ALL) and in many adult ALL patients. MRD diagnostics has proven to be the strongest prognostic factor, allowing for risk group assignment into different treatment arms, ranging from significant treatment reduction to mild or strong intensification. Also in relapsed ALL patients and patients undergoing stem cell transplantation, MRD diagnostics is guiding treatment decisions. This is also why the efficacy of innovative drugs, such as antibodies and small molecules, are currently being evaluated with MRD diagnostics within clinical trials. In fact, MRD measurements might well be used as a surrogate end point, thereby significantly shortening the follow-up. The MRD techniques need to be sensitive ($\leq 10^{-4}$), broadly applicable, accurate, reliable, fast, and affordable. Thus far, flow cytometry and polymerase chain reaction (PCR) analysis of rearranged immunoglobulin and T-cell receptor genes (allele-specific oligonucleotide [ASO]-PCR) are claimed to meet these criteria, but classical flow cytometry does not reach a solid 10^{-4}, whereas classical ASO-PCR is time-consuming and labor intensive. Therefore, 2 high-throughput technologies are being explored, ie, high-throughput sequencing and next-generation (multidimensional) flow cytometry, both evaluating millions of sequences or cells, respectively. Each of them has specific advantages and disadvantages. (*Blood*. 2015;125(26):3996-4009)



Leukemia 2002; 16 (8):1432-36 van de Velden et all.
MRD levels in BM and PB are comparable in T cell ALL but not in precursor B ALL

Asignación de grupos de riesgo (GR) en LLA pediátrica- ALLIC

Características biológicas al Dx

- Edad
- WBC
- Alteraciones citogenéticas y/o moleculares

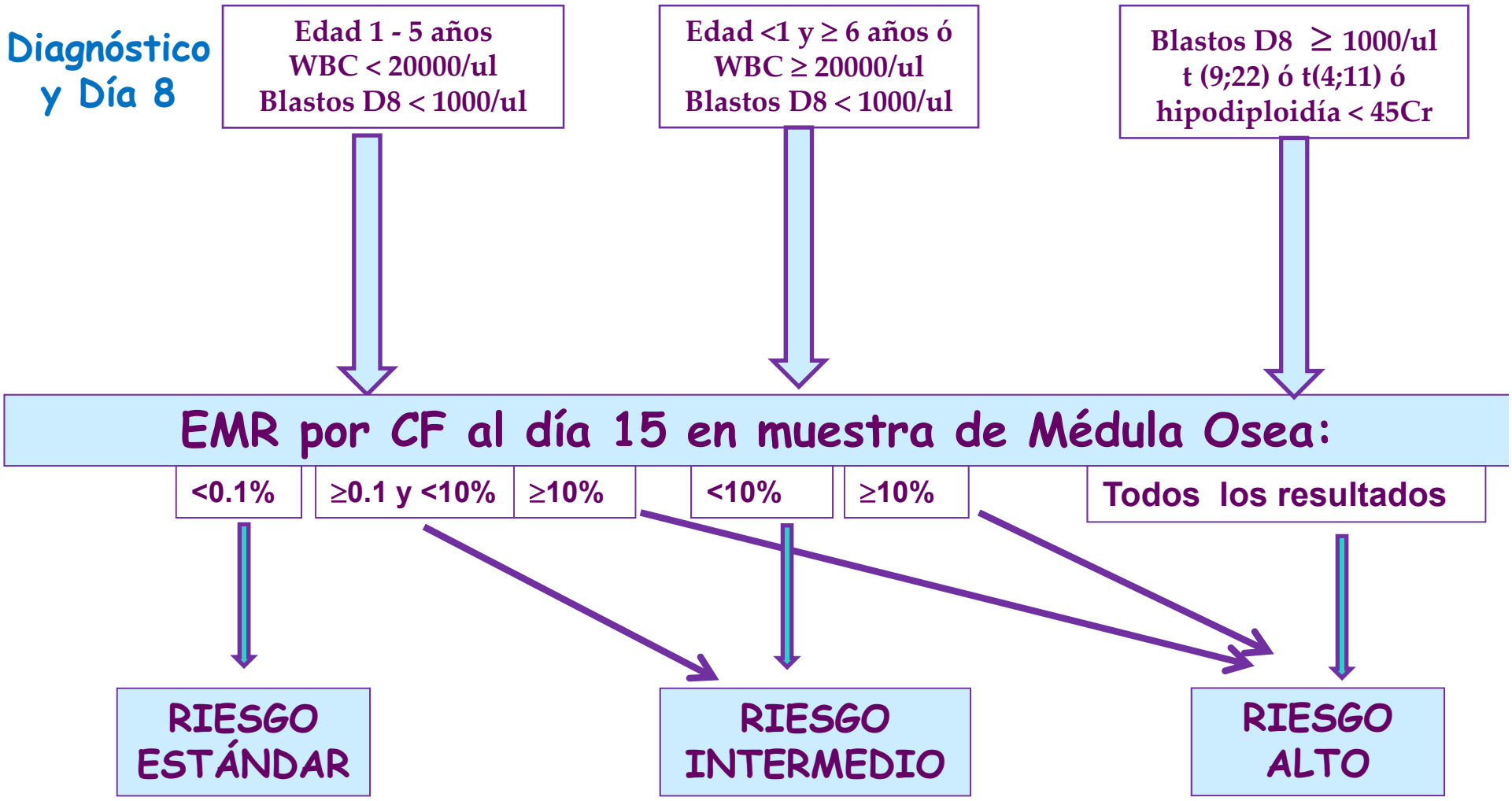
Respuesta temprana al tratamiento

- Rta. a la prednisona en SP
- MO día 15

-Protocolo ALLIC-2002
Morfológica
(<5%, 5-25%, >25%)

- Protocolo ALLIC-2009
EMR por CF al Día 15
(<0.1%, 0.1-10%, >10%)

Estratificación en grupos de riesgo ALLIC 2009



CONSIDERACIONES IMPORTANTES para el uso clínico de la EMR en protocolos ALLIC

Muestra: M. ósea del día 15.

Utilidad clínica: Estratificación en grupos de riesgo.

Etapa del tratamiento:

Día 15 para la estratificación en grupos de riesgo en protocolos ALLIC.

En otros momentos del tratamiento solo puede confirmar o descartar la presencia de blastos.

D33 y D78:En el próximo ALLIC se incluirá también.

APLICACIONES CLÍNICAS DE LA EMR

VOLUME 27 · NUMBER 31 · NOVEMBER 1 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Risk of Relapse of Childhood Acute Lymphoblastic Leukemia Is Predicted By Flow Cytometric Measurement of Residual Disease on Day 15 Bone Marrow

Giuseppe Basso, Marinella Veltroni, Maria Grazia Valsecchi, Michael N. Dworzak, Richard Ratei, Daniela Silvestri, Alessandra Benetello, Barbara Buldini, Oscar Maglia, Giuseppe Masera, Valentino Conter, Maurizio Arico, Andrea Biondi, and Giuseppe Gaipa

From the Laboratorio di Oncoematologia Pediatrica, Department of Pediat-

See accompanying editorial on page 5121 and articles on pages 5175 and 5189

OBJETIVO

Evaluar el impacto pronóstico de la determinación de EMR por CF al día 15 en los pacientes de AEIOP-BFM 2000 en los que se asignó grupo de riesgo por BM D33 y D78 (gold standard de estratificación).

Determinación de los valores de EMR CF para la asignación de GR

% ptes.	% EMR	SLE 5 años	IAR 5 años	GR
42%	<0.1%	89.9 %	7.5%	Estandar
47%	0.1-10%	79.3 %	17.2%	Intermedio
11%	≥10%	46.1 %	47.2%	Alto

EMR por CF al día 15 en MO

Identificar pacientes con alto y bajo riesgo de recaída

Ajustar la intensidad del tto para evitar la recaída

- ✓ EMR al Dia 15 por CF factor pronóstico independiente más importante disponible en este momento del tto.

- ✓ En un análisis multivarianza la EMR por CF al día 15 ≥10% resultó ser un factor de riesgo de recaída independiente aún en los pacientes que habían sido estratificados en base a la EMR por BM al día 33 y 78.

Protocolo BFM-ALLIC-2009
(Acute Lymphoblastic Leukemia Intercontinental 2009)

EMR por CF es incorporada como criterio de estratificación sólo después de que haya sido estandarizada y evaluada para los laboratorios participantes.

PAUTAS ALLIC 2009

PAUTAS BFM-ALLIC-2009 para obtener la habilitación del laboratorio para EMR-CF

***Monitoreo Intralaboratorio:**

-Control estricto de calibración y mantenimiento del citómetro.

-Estandarización de metodologías.

***Monitoreo Interlaboratorios:**

-Entrenamiento: Transferencia de pautas de trabajo en el laboratorio sponsor AIEOP-BFM

-Control de calidad:

I.-Los laboratorios participantes deben completar exitosamente 2 rondas anuales de análisis de datos

II.- Control de calidad externo UK-NEQAS.

III.-Control de resultados de EMR en laboratorio sponsor:

-Envío copia del análisis y resultado de la EMR D15.

-Evaluación por el sponsor.

-Conclusión final del análisis y ratificación del resultado.

Pautas BFM-ALLIC 09. Marcación y adquisición.

- Muestra: Médula ósea del día 15.
- Marcación y adquisición:
  - Marcación seguida de lisis (salvo en casos de hipocelularidad extrema donde se lisa primero).
  - Adquirir al menos 300.000 células nucleadas (cél syto +), y que representen el 30% de la celularidad de la médula (%syto+ ≥30%)
  - Cargar al menos 700,000 céls/tubo.

  - **Nosotros normalmente marcamos ≥2x10^6 cél para adquirir ≥1x10^6 cels.**

Criterios evaluabilidad para EMR d15 según BFM-ALLIC-2009

1. Eritroblastos >2%
2. Syto+ >30% y >300000 células nucleadas (ideal)
3. Cluster EMR ≥30 células (30 cél patológicas en 300000 cel nucleadas para alcanzar la sensibilidad de 0,01% requerida para la EMR, **o ≥30 eventos en 30000 células nucleadas pero con sensiblidad 0.1%**

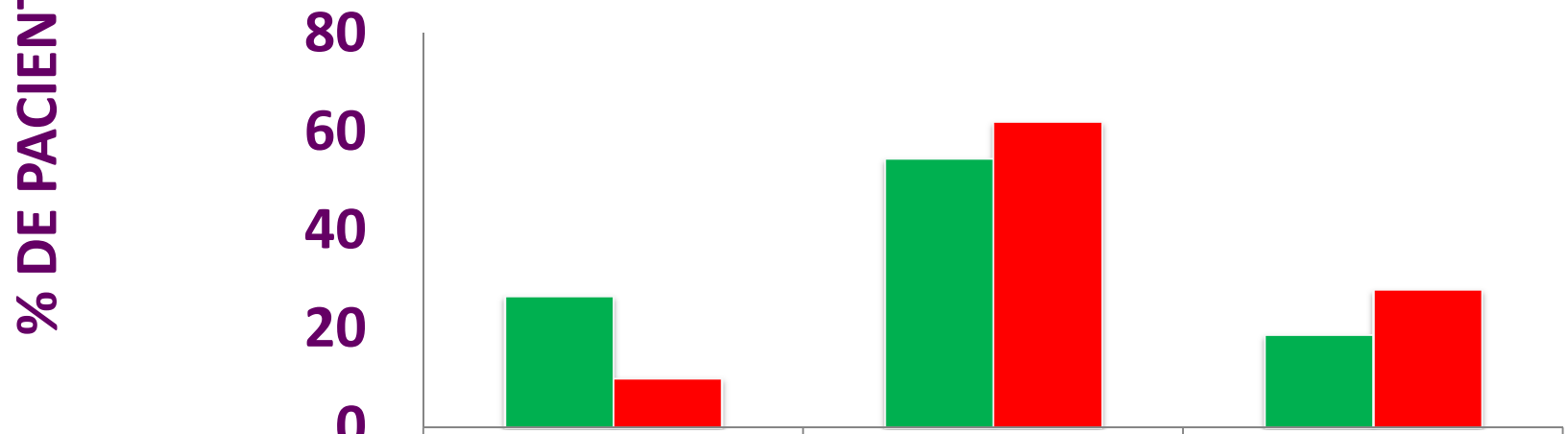
Reporte

- Identificación del paciente
- Panel utilizado
- # y % células nucleadas
- % eritroblastos
- % EMR.
- Si hay discrepancia en el valor entre los tubos, se reporta el valor más alto del tubo donde se ve con mayor claridad la población.

Nuestra experiencia

EMR POR CITOMETRIA DE FLUJO AL DIA 15

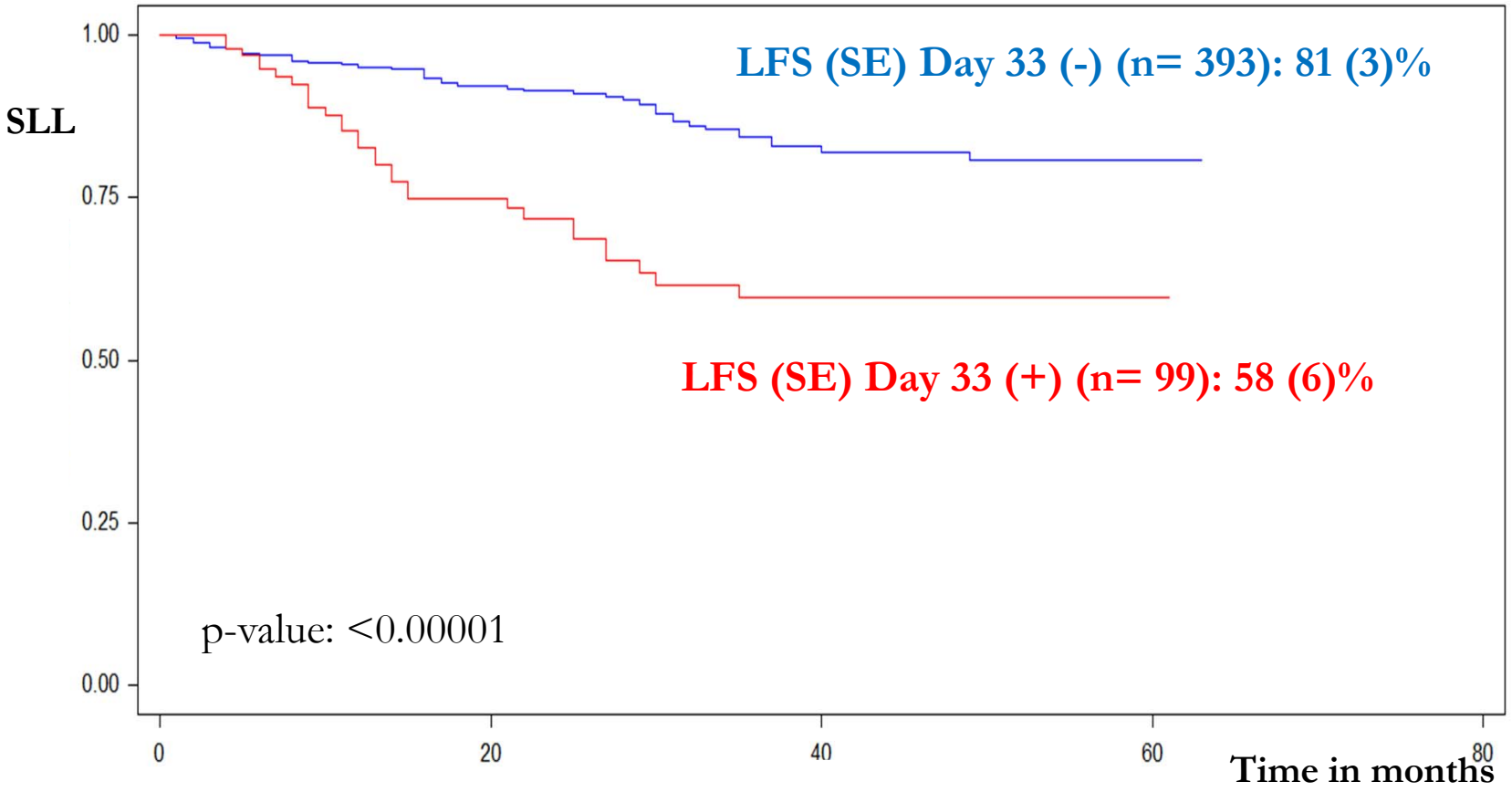
IMPACTO DE LA INCORPORACION DE EMR AL DIA 15



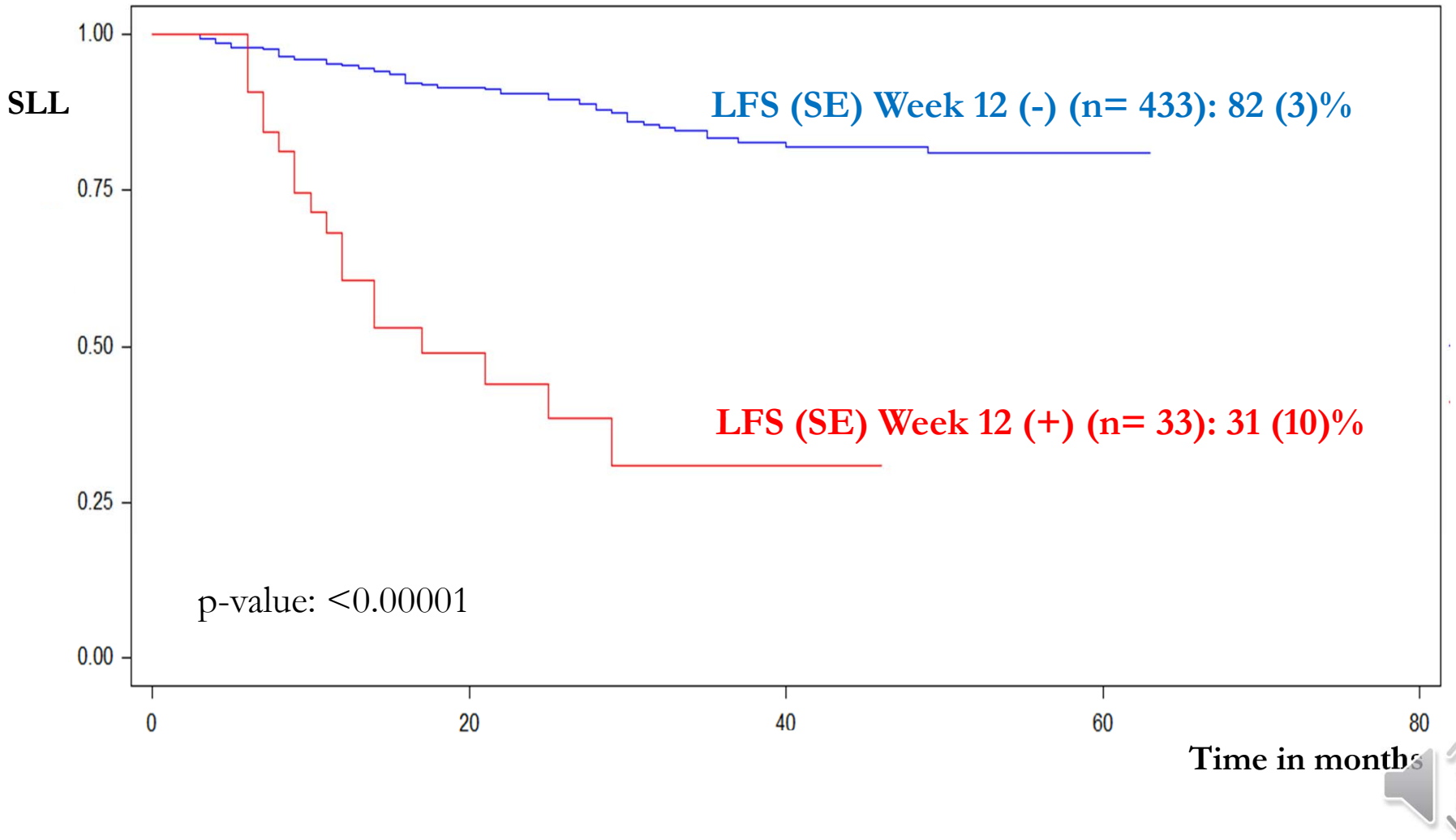
	RE	RI	RA
PRE DIA 15 (%)	26.7	54.5	18.8
POST DIA 15 (%)	10	62	28

26% DE LOS PACIENTES FUERON REASIGNADOS A UN GRUPO DE MAYOR RIESGO

SLL (sobrevida libre de leucemia) según la EMR al día 33 (+ vs -) (n= 492)



SLL (sobrevida libre de leucemia) según la EMR al día 78 (+ vs -) (n= 466)



CONCLUSIONES

La EMR por CF:

- ✓Al Dia 15 permite la reasignación del grupo de riesgo en aproximadamente 20% de los pacientes tratados en protocolos del ALLIC-BFM.
- ✓Es indispensable para la correcta adecuación del tratamiento en los protocolos actuales.
- ✓La evaluación en tiempos posteriores (día 33 y día 78) también brinda información clínicamente relevante.
- ✓Ídem en protocolos de LLA recaída

Otras aplicaciones clínicas de la EMR

- Está probada su utilidad para **identificar los pacientes de muy bajo riesgo de recaer y ellos beneficiarse con una reducción en la intensidad del tratamiento para evitar toxicidad innecesaria (UKALL, Total St Jude)**
- EMR pre Tx para determinar si necesita mayor tiempo de QT u otra alternativa terapéutica. **Lo ideal es el paciente con EMR negativa pre Tx.**
- La EMR post trasplante de médula ósea brinda información sobre el riesgo de nueva recaída.

Otras aplicaciones clínicas de la EMR

- **EMR en LMA pediátrica.**
  - En los últimos 5 años se publicaron series de pacientes donde demuestran su utilidad como factor pronóstico y de adecuación de tratamiento.
  - Es altamente compleja por su gran heterogeneidad por la presencia de varias subpoblaciones de clones leucémicos al diagnóstico los cuales responden distinto al tratamiento y en cuanto a la variación en la expresión de ciertos marcadores.
  - Nuevas publicaciones identificando los marcadores mas adecuados para EMR en LMA
  - La CF sensibilidad de 0.1% actualmente para EMR en LMA

bjh research paper

Prognostic significance of flow-cytometry evaluation of minimal residual disease in children with acute myeloid leukaemia treated according to the AIEOP-AML 2002/01 study protocol

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bjh BRITISH JOURNAL OF HAEMATOLOGY

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Summary

In children with acute myeloid leukaemia (AML), assessment of initial treatment response is an essential prognostic factor; methods more sensitive than morphology are still under evaluation. We report on the measurement of minimal residual disease (MRD), by multicolour flow-cytometry in one centralized laboratory, in 142 children with newly diagnosed AML enrolled in the Associazione Italiana di EmatoOncologia Pediatrica-AML 2002/01 trial. At the end of the first induction course, MRD was <0·1% in 69, 0·1–1% in 16 and >1% in 51 patients. The 8-year disease-free survival (DFS) of 125 children in morphological complete remission and with MRD <0·1%, 0·1–1% and ≥1% was 73·1 ± 5·6%, 37·8 ± 12·1% and 34·1 ± 8·8%, respectively ($P < 0·01$). MRD was also available after the second induction course in 92/142 patients. MRD was ≥0·1% at the end of the first induction course in 36 patients; 13 reached an MRD <0·1% after the second one and their DFS was 45·4 ± 16·7% vs. 22·8 ± 8·9% in patients with persisting MRD ≥0·1% ($P = 0·037$). Multivariate analysis demonstrated that MRD ≥0·1% after first induction course was, together with a monosomal karyotype, an independent adverse prognostic factor for DFS. Our results show that MRD detected by flow-cytometry after induction therapy predicts outcome in patients with childhood AML and can help stratifying post-remission treatment.

Keywords: Acute myeloid leukaemia, Minimal residual disease, Flow-cytometry, Paediatric; Risk group.

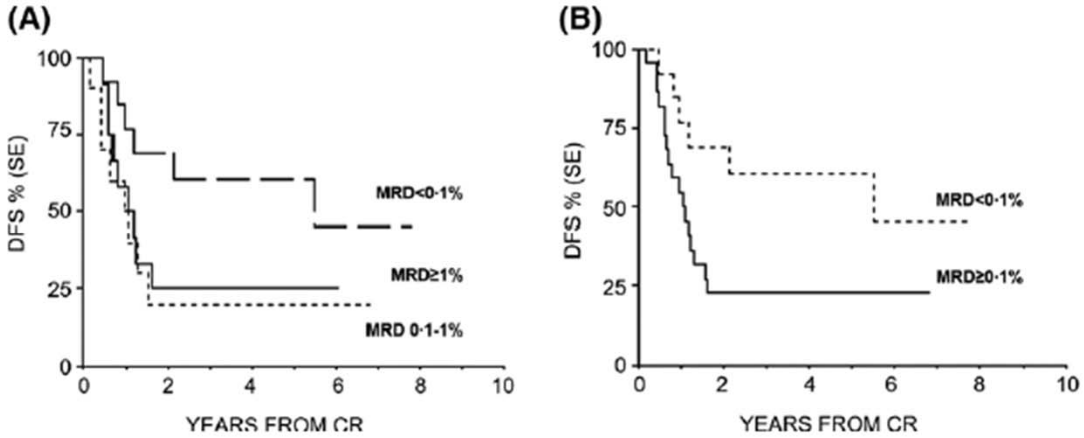

Fig 4. DFS by MFC-MRD status at the end of induction 2, in patients with MRD ≥0·1% at the end of induction 1. According to MRD levels after the second course of chemotherapy, patients were stratified into three MRD groups (MRD ≤0·1%; MRD 0·1–1%; MRD ≥1%) (A) or into two MRD groups (MRD ≤0·1%; MRD ≥0·1%) (B). CR, complete remission; DFS, disease-free survival; MFC, multi-colour flow cytometry; MRD, minimal residual disease; SE, standard error.

How close are we to incorporating measurable residual disease into clinical practice for acute myeloid leukemia?

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Muchas gracias

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